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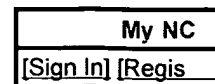
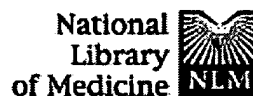
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Acceptor specificity of GDP-Fuc:Gal beta 1-->4GlcNAc-R alpha 3-fucosyltransferase VI (FucT VI) expressed in insect cells as soluble, secret enzyme.

De Vries T, Palcic MP, Schoenmakers PS, Van Den Eijnden DH, Joziassse DH.

Department of Medical Chemistry, Vrije Universiteit Amsterdam, The Netherlands.

As an extension of previous study (de Vries et al., 1995, J. Biol. Chem., 270, 8712-8722) the acceptor specificity of recombinant FucT VI, expressed in insect cells as soluble enzyme, and purified from the growth medium by affinity chromatography, was analyzed toward a broad panel of oligosaccharide and glycoprotein substrates. It was found that FucT VI effectively utilizes any type-2-chain based structure (Gal beta 1-->4GlcNAc-R) Neutral as well as sialylated structures are fucosylated with high efficiency. To identify polar groups on acceptors that function in enzyme binding, deoxygenated substrate analogs were tested as acceptors. FucT VI had an absolute requirement for a hydroxyl at C-6 of galactose in addition to the accepting hydroxyl at C-3. Thus, FucT VI, although different from FucT III, IV, and V in acceptor properties, seems to bind the acceptor in a similar way.

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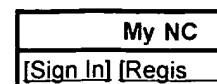
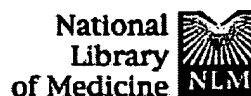
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Production of soluble human alpha3-fucosyltransferase (FucT VII) by membrane targeting and in vivo proteolysis.

de Vries T, Storm J, Rotteveel F, Verdonk G, van Duin M, van den Eijnden DH, Joziase DH, Bunschoten H.

Department of Medical Chemistry, Vrije Universiteit Amsterdam, Van der Boechorststra 7, 1081 BT Amsterdam, The Netherlands.

The rational design of fucosyltransferase (FucT VII) inhibitors as potential medication in the treatment of rheumatoid arthritis requires the three-dimensional structure of this member of the glycosyltransferase family. Structure determination by X-ray diffraction analysis needs purified, soluble enzyme protein. For this purpose we developed a novel method for the high-yield production of soluble FucT VII by in vivo proteolysis. To obtain a soluble form of FucT VII a mammalian expression construct was made encoding an N terminal portion of FucT VI (amino acids 1-63) fused with the stem region and catalytic domain of FucT VII (amino acids 39-342). Chinese hamster ovary cells stably transfected with this construct produced FucT activity in the supernatant, which has the same catalytic properties as wild-type FucT VII. This soluble form of FucT VII can be obtained in high amounts (1 mg/L) and can be efficiently purified by GDP-hexanolamine affinity chromatography. In conclusion, it was demonstrated that the intrinsic properties of FucT VII could be transferred to secreted FucT VII constructs, which may open possibilities for production of soluble forms of other members of the glycosyltransferase family as well.

PMID: 11555615 [PubMed - indexed for MEDLINE]

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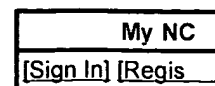
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Acceptor specificity of different length constructs of human recombinant alpha 1,3/4-fucosyltransferases. Replacement of the stem region and the transmembrane domain of fucosyltransferase V by protein A results in an enzyme with GDP-fucose hydrolyzing activity.

de Vries T, Srnka CA, Palcic MM, Swiedler SJ, van den Eijnden DH, Macher BA.

Department of Chemistry and Biochemistry, San Francisco State University, California 94132, USA.

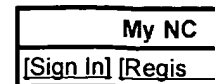
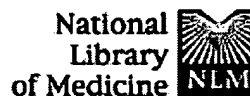
The acceptor specificity of recombinant full-length, membrane-bound fucosyltransferase expressed in COS-7 cells, and soluble, protein-A chimeric forms of alpha 1,3-fucosyltransferase (Fuc-T) III, Fuc-TIV, and Fuc-TV was analyzed toward a broad panel oligosaccharide, glycolipid, and glycoprotein substrates. Our results on the full-length enzymes confirm and extend previous studies. However, chimeric Fuc-Ts showed increased activity toward glycoproteins, whereas chimeric Fuc-TIII and Fuc-TV had a decreased activity with glycosphingolipids, compared to the full-length enzymes.

Unexpectedly, chimeric Fuc-TV exhibited a GDP-fucose hydrolyzing activity. In substrata with multiple acceptor sites, the preferred site of fucosylation was identified. Fuc-TIII and Fuc-TV catalyzed fucose transfer exclusively to OH-3 of glucose in lacto-N-neotetraose and lacto-N-tetraose, respectively, as was demonstrated by ¹H NMR spectroscopy. Thin layer chromatography immunostaining revealed that Fuc-TIV preferred the distal GlcNA residue in nLc6Cer, whereas Fuc-TV preferred the proximal GlcNAc residue. Incubation of Fuc-TIV or Fuc-TV with VI3NeuAcnLc6Cer resulted in products with the sialyl-LewisX epitope as well as the VIM-2 structure. To identify polar groups on acceptors that function in enzyme binding, deoxygenated substrate analogs were tested as acceptors. A three Fuc-Ts had an absolute requirement for a hydroxyl at C-6 of galactose in addition to the accepting hydroxyl at C-3 or C-4 of GlcNAc.

PMID: 7721776 [PubMed - indexed for MEDLINE]

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Purification and separation of two soluble fucosyltransferase activities of small intestinal mucosa.

Martin A, Biol MC, Richard M, Louisot P.

Department of General and Medical Biochemistry, INSERM-CNRS U189, Lyon-Sud Medical School, Oullins, France.

1. Rat small intestinal soluble fucosyltransferase is purified more than 2000-fold using chromatographic procedures with DEAE-cellulose, CM-cellulose, GDP-Sepharose and Concanavalin A-Sepharose. 2. Chromatography on Sephadex G15 of the final enzymatic fraction clearly separates two activities: a first peak incorporates fucose on asialoserotransferrin and a second peak on asialofetuin. 3. The use of small saccharidic acceptors (phenylgalactose, lactose, lacto-N-fucopentaose I) and the analysis of fucosylated asialoglycoproteins indicate that the first activity corresponds to an alpha-(3/4)-fucosyltransferase and the second one to an alpha-(1-2)-fucosyltransferase. 4. Protein analysis by polyacrylamide gel electrophoresis in the presence of SDS for each enzyme shows two bands corresponding to a mol. wt of about 65,000 and 70,000. The two enzymes have the same sensitivity to the action of N-ethylmaleimide.

PMID: 3665424 [PubMed - indexed for MEDLINE]

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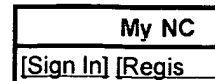
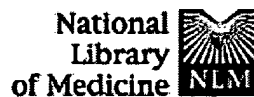
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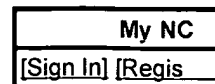
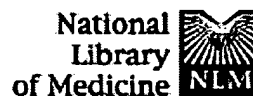
An amino acid region at the N-terminus of rat hepatoma alpha1-->2 fucosyltransferase modulates enzyme activity and interaction with lipids: strong preference for glycosphingolipids containing terminal Galbeta1-->3GalNAc-structures.

Sherwood AL, Stroud MR, Lavery SB, Holmes EH.

Department of Molecular Medicine, Northwest Hospital, 21720 23rd Drive SE, Suite 10 Bothell, Washington 98021, USA. asherwoo@mmnwh.org

A GDP-fucose:GM1 alpha1-->2 fucosyltransferase (FucT) is induced during early stage chemical hepatocarcinogenesis in parenchymal cells of Fischer 344 rats fed a diet supplemented with 0.03% N-2-acetylaminofluorene (AAF). This enzyme is undetectable normal rat liver tissues but is highly expressed in many rat hepatoma cell lines, including rat hepatoma H35 cells. Enzymatic properties and acceptor specificity of native rat hepatoma H35 cell alpha1-->2FucT, expressed recombinant full-length H35 cell alpha1-->2FucT, and a truncated form missing the first 27 amino acid residues from the N-terminus, comprising the cytoplasmic and transmembrane domains of the enzyme, were studied. The results indicate that the recombinant full-length enzyme has a specific activity over 80-fold higher than the truncated enzyme. Both the native and recombinant full-length enzymes display significant activity in the absence of detergent or phospholipid and optimal activity in the presence of Triton CF-54 detergent. The truncated enzyme is optimally activated by CHAPSO, showing little activity in its absence. These findings are in agreement with previous studies demonstrating a requirement of a lipidic environment for optimal activity with this enzyme and suggest that the N-terminal transmembrane domain is important either in the maintenance of an active conformation or in allowing efficient interaction with acceptor glycolipids. Both the full-length and truncated enzyme transfer fucose not only to GM1 and asialo-GM1 (Gg4) but also to galactosyl globoside (Gb5) as well. Weak or undetectable transfer to lacto- and neolacto-series acceptors was observed, demonstrating a strong preference for terminal Galbeta1-->3GalNAc- structures. The structures of two reaction products generated by expressed recombinant full-length alpha1-->2FucT, which are known to be important tumor-associated antigens (fucosyl-GM1 and fucosyl-Gb5), were unambiguously confirmed by 1H-NMR spectral analysis.

PMID: 11341836 [PubMed - indexed for MEDLINE]



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- Expression of functional soluble forms of human beta-1, 4-galactosyltransferase I, alpha 2,6-sialyltransferase, and alpha-1, 3-fucosyltransferase VI in the methylotrophic yeast *Pichia pastoris*.
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- Effects of endogenous soluble beta-galactoside binding lectins and protein inhibitor of fucosyltransferase on the enzymes involved in the intestinal fucosylation process.
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